www.ijarp.com ISSN 2456-9992 Page: 01-10

International Journal Advanced Research Publications

Research Article

Volume: 02

Issue: 11

ORANGE PEEL AS A SOURCE OF BIOACTIVE COMPOUNDS: ANTIMICROBIAL PROPERTIES, GUT HEALTH BENEFITS AND PHARMACEUTICAL USES

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Article Received: 12 October 2026, Article Revised: 01 November r 2026, Published on: 20 November 2026

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Doi: https://doi-doi.org/101555/ijarp.7900

ABSTRACT

Orange peel is a byproduct of juice production and home use. It is a source of essential oils, flavanones, polymethoxyflavones, phenolic acids, pectin and a whole range of other dietary fibre sources with potential in the pharma industry. This narrative review provides an overview of the composition of orange peel and its potential therapeutic applications. The antimicrobial potential of the bioactives will be discussed, along with a look at the methods used to assess this activity (agar diffusion, broth dilution, agar dilution, MIC, MBC, time-kill, biofilm assays, synergy studies and more advanced techniques). Agar diffusion assays can be useful for screening. It will not give any information on the potency of the sample and this can be a problem with hydrophobic essential oils and/or coloured or viscous samples. Quantitative methods will require the use of a defined inoculum, appropriate media, appropriate solvent controls, reference strains and a clear endpoint. Orange peel is a source of pectin and pectooligosaccharides as well as polyphenols which can be metabolised by the gut microbiota. The bulk of the evidence for orange peel is pre-clinical. The purpose of this review is to highlight the opportunities that orange peel presents (standardised extracts, antimicrobial formulations, pectin based materials, encapsulation systems and green nanomaterials). The composition will vary depending on the cultivar, type of peel, ripeness, processing and the extraction method. Standardisation, safety, bioavailability and designed clinical studies are all essential before any claims can be made as to the therapeutic

potential of orange peel. In vitro studies have demonstrated activity but this does not necessarily equate to clinical efficacy.

KEYWORDS: Orange peel; Citrus sinensis; Flavonoids; activity; Gut microbiota; Pectin; Pharmaceutical opportunities.

1. INTRODUCTION

Orange (Citrus sinensis (L.) Osbeck) is by far the most consumed of the citrus fruits. The amount of orange peel generated in the course of domestic use and juice production is considerable. Orange peel is a source of a range of chemical constituents including volatile terpenes, flavonoids, phenolic acids, carotenoids and structural polysaccharides. These are all of interest in the fields of pharmacognosy, natural products, microbiology and pharmaceutical sciences. It is a source of antimicrobial leads, fermentable fibres and formulation materials [1-4].

Orange peel consists of two parts: the flavedo (the coloured part) and the albedo (the inner white part). The flavedo contains numerous oil glands that store essential oils, in which D-limonene is generally the major volatile constituent of sweet orange peel. The albedo is rich in pectin and other cell wall polysaccharides. Flavanones (hesperidin and narirutin) are present throughout the peel. Some of the polymethoxyflavones (nobiletin and tangeretin) are also present. The relative amounts of each of these constituents will vary depending on the cultivar the environment, the degree of ripeness the storage conditions, the drying and the extraction method [2-5].

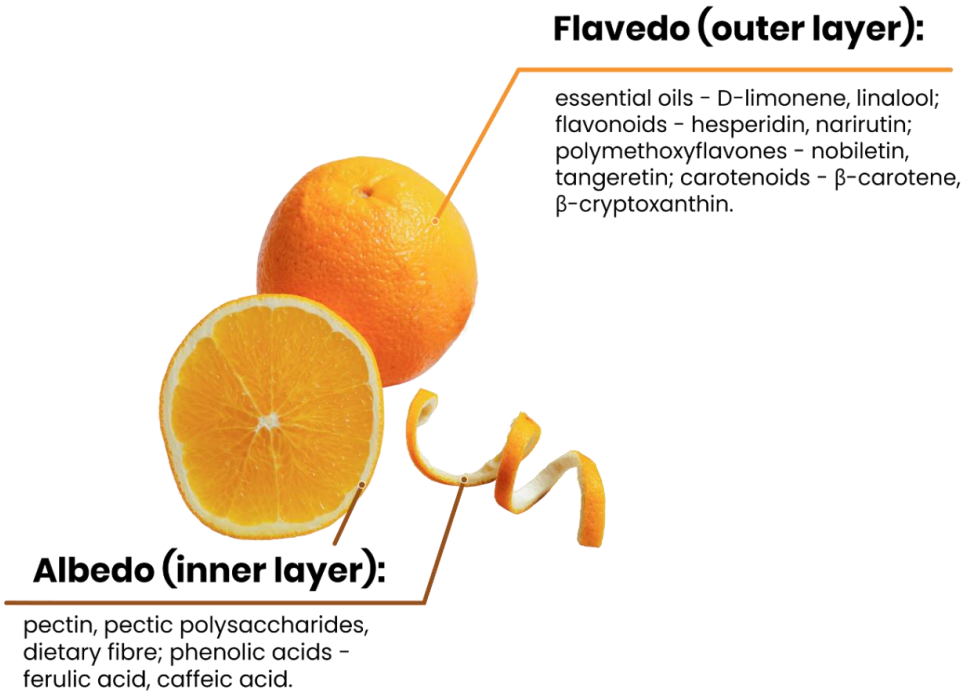

Figure 1. Sweet Orange Peel Structure and Bioactive Compounds.

There has been a bit of a surge in interest in orange peel as a result of antimicrobial resistance and the search for sustainable sources of bioactives. A number of in vitro studies have demonstrated the ability of orange peel extracts to inhibit a range of Gram-negative and fungal species. The variation between studies in terms of the solvent used for extraction, the organism tested, the concentration of extract and the method of testing is considerable. Inhibition zones are not comparable between the studies [6-9].

The fibres extracted from citrus also have an effect on the gut microbiota. The pectin and pectooligosaccharides will be fermented by the gut microbes. This will in turn affect the production of short chain fatty acids and the composition of the microbiota. Orange peel is also a source of polyphenols which will reach the colon and be metabolised by the population. That said, it must be noted that much of the evidence is from in vitro fermentation, animal studies or with other citrus species. [10-13].

This review will cover the phytochemistry, microbiology, GI health and pharmaceutical aspects. A significant portion of the review will be devoted to the methods used to assess activity.

2. Phytochemical Composition and Bioactive Constituents of Orange Peel

2.1 Main constituents

The essential oils are predominantly located in the oil glands of the flavedo. D-limonene is generally the volatile component in sweet orange peel oil. Smaller quantities of myrcene, alpha-pinene, sabinene, linalool, aldehydes and a number of terpenoids are also present. The make-up will vary according to cultivar and method of extraction. Volatiles can be lost during drying and prolonged heating so the processing method must be recorded. [3,4,14]

The major non-volatile constituents are the flavanones. Hesperidin, narirutin, naringin, didymin and various other glycosides are present in the peel of citrus. Hesperidin is the glycoside of hesperetin. Narirutin and naringin are the glycosides of naringenin. The aglycones can be liberated by hydrolysis using either the enzymes in the intestine or the gut microflora. The polymethoxyflavones (nobiletin, tangeretin and sinensetin) are relatively lipophilic. They have of course been of interest for their anti-inflammatory activity. [2,5,15]

Acids (caffeic, ferulic, p-coumaric and sinapic) are all present. Carotenoids, vitamin C, limonoids and a number of minor constituents may also be found. Pectin, cellulose and hemicellulose are the structural constituents. There is variation in the composition of citrus peel. [2,3,5]

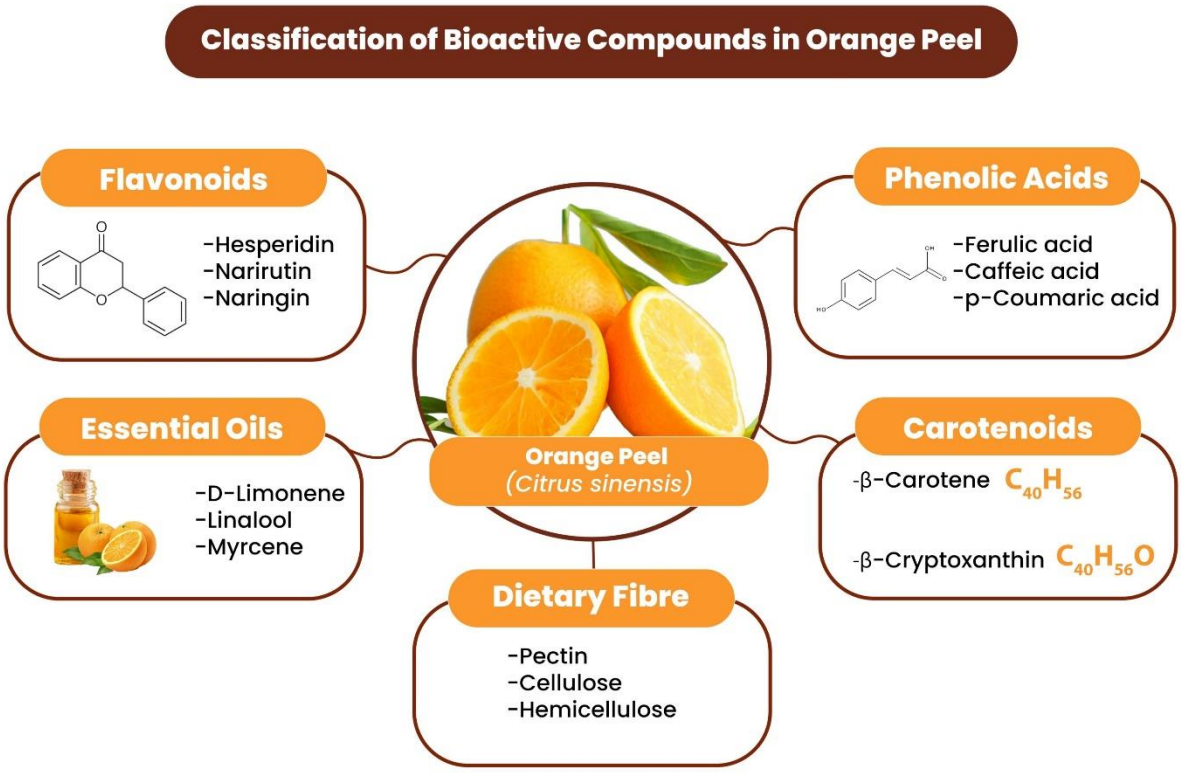

Figure 2 – Classification of Bioactive Compounds in Orange Peel.

2.2 Influencing factors

Cultivar, location, season of availability, ripeness, type of peel presence of pesticides, post-harvest handling, water content and storage all have an effect on the chemical profile. There can be a difference between fresh orange peel due to the loss of volatile constituents. One study looked at the ethanol extraction of fresh and dry C. sinensis peel. The fresh peel had higher levels of total phenolics, flavonoids and tannins. The fresh peel also demonstrated activity against a range of bacterial species. The trend was not always the same with the moulds though. [6] This demonstrates that there is not necessarily a single optimal extraction method.

2.3 Extraction and characterisation

Traditional techniques are maceration, percolation, soxhlet, hydrodistillation and cold pressing. Modern techniques are ultrasound, microwave, pressurised liquid and supercritical fluid extraction. The solvent employed will dictate the type of compounds that are extracted. Non-polar solvents will extract non-polar compounds. Hydrodistillation is the method of choice for the extraction of oils. Green extraction is about reducing the toxicity of the solvent, reducing energy consumption and reducing the environmental impact. [3,4,16]

A standardised extract should be described. This would include the name, the part of the plant used, the source, how it was collected and processed, the solvent, the ratio, the time, the temperature, the yield, the storage conditions and the chemical fingerprint. Total phenolic and flavonoid assays can be used to give an idea of the levels but will not give the levels of any particular constituent. HPLC, UPLC, LC-MS and GC-MS can be used to give specific information. GC-MS can be used for oils and HPLC/LC-MS for the flavonoids and phenolic acids. [3,5,6]

3. BIOACTIVE COMPOUNDS AND ANTIMICROBIAL POTENTIAL

3.1 Essential oil and D-limonene

Orange peel essential oil is a hydrophobic mixture rather than a single active ingredient. D-limonene is likely to be the agent responsible for the antimicrobial effect via interaction with the microbial membrane, alteration of membrane permeability and consequent leakage of intracellular contents. Other terpenes may be acting additively or synergistically. The degree of activity will be a function of the oil composition, the microorganism in question, the method of dispersion, the volatility and the test system. The oils will tend to partition very poorly into aqueous broth and will diffuse quite unevenly through agar so if the formulation is not very carefully controlled artefacts are very easily generated [7,8,14].

3.2 Flavanones and polymethoxyflavones

Citrus flavonoids have been investigated for antibacterial and antifungal activity with proposed mechanisms of action including interaction with cell-envelope proteins, disruption of membrane function, enzyme inhibition, metal chelation, interference with energy metabolism and modulation of virulence-related processes. The evidence is very much compound and organism specific. The presence of hesperidin or naringin in an extract does not necessarily mean that either of those compounds is the one responsible for the zone of inhibition observed. The crude extracts contain a whole host of compounds and will require bioassay guided fractionation to identify the active principle(s) [5,15].

3.3 Phenolic acids and tannins

Phenolics can have an effect on membrane integrity, redox balance and the enzymes involved in microbial metabolism. Tannins can bind to proteins and macromolecules and so may be interfering with cell wall associated proteins or extracellular enzymes. These are the sorts of mechanisms that are generally put forward on the basis of in vitro work and should not be considered as being applicable to all orange peel preparations. The antioxidant activity observed in a chemical radical scavenging assay is not the same as antimicrobial activity and both should be measured separately.

3.4 Antibacterial and antifungal evidence

Oikeh and co-workers looked at the antimicrobial activity of ethanol extracts of both fresh and dried sweet orange peel against a range of bacteria (S. aureus, E. faecalis, P. aeruginosa, E. coli and S. Typhimurium) and fungi (C. albicans, A. niger and P. notatum) using the diffusion method. The results

were organism dependent and there were differences between the fresh and dried extracts. The study does support further work but does not establish any clinical efficacy or a broad spectrum of activity [6].

Other citrus flavonoid studies and reviews have reported antimicrobial activity against a range of micro-organisms. It is crucial to point out that results obtained with purified flavonoids, essential oils, other citrus species or in food preservation systems should be clearly distinguished from those obtained with a standardised C. sinensis peel extract [5,15]. Future work should report strain IDs, extract composition, concentration units, solvent controls and quantitative MIC/MBC or MFC values.

3.5 Proposed mechanisms of antimicrobial action

The mechanisms involved are all entirely plausible and could well work in combination: (i) membrane disruption and increased permeability; (ii) leakage of K+, nucleotides and proteins; (iii) interference with membrane bound enzymes and the proton motive force; (iv) inhibition of certain metabolic enzymes; (v) binding/inactivation of proteins/enzymes by the polyphenols; (vi) induction of oxidative or redox stress in certain instances; and (vii) It is interference, with adhesion, quorum sensing or biofilm formation. These are ideas that should be tested with membrane-integrity dyes, leakage tests, microscopy, ATP checks, enzyme tests and gene-expression studies instead of just guessing from inhibition zones.

Table 1. Orange Peel Components and Antimicrobial Roles.

Component	Potential antimicrobial role	Evidence status / caution
D-limonene	May disrupt microbial cell membranes	Mainly in vitro evidence; activity depends on concentration
Linalool	May interfere with microbial membrane integrity	Requires organism-specific evaluation
Hesperidin	Potential effects on microbial growth and metabolism	Evidence varies with experimental conditions
Naringin	Potential antimicrobial and antioxidant effects	Bioavailability and effective concentrations require clarification
Nobiletin	Potential modulation of microbial growth	Limited direct evidence for orange-peel preparations

4. PRINCIPLES AND PROCEDURES OF MAJOR ANTIMICROBIAL METHODS.

4.1 Agar disc diffusion

Principle: A paper disc containing a known quantity of the test material is placed onto an agar surface which has been inoculated with a standardised microbial suspension. The agent will diffuse out radially and may produce a zone of inhibition in which growth is visibly inhibited. The diameter of the zone is measured in mm after incubation. It is an inexpensive method and can be useful for a first comparison of different extracts or fractions.

Procedure: Take a pure, fresh culture, standardise the inoculum, uniformly inoculate an appropriate agar plate, apply the discs containing a known volume and concentration of the test substance, include solvent only and reference antimicrobial controls, incubate under the organism-appropriate conditions and measure the full inhibition diameter. For plant extracts, mass per disc and loading volume should be reported. A zone must be read in conjunction with the disc diameter and the controls.

Interpretation and limitations: larger zones may indicate greater apparent activity under the specific conditions, but zone size is dependent on diffusion, solubility, molecular size, agar depth, inoculum and concentration. Hydrophobic limonene-rich oils and viscous or pigmented extracts will diffuse poorly. The clinical susceptible/intermediate/resistant categories and antibiotic breakpoints are not valid for crude botanical extracts unless a dedicated validated breakpoint system is in place. Disc diffusion is therefore a screening method and not a measure of clinical potency in itself [16,17,19].

4.2 Agar well diffusion

Principle and procedure: wells are punched into the inoculated agar and filled with a measured volume of extract. The compound then diffuses from the well into the agar and the zone is measured after incubation. This format is advantageous in that it allows for larger sample volumes than the disc format and can be useful with crude extracts. The results are, however, susceptible to a number of variables including well diameter, agar thickness, volume, precipitation and diffusion rate. Wells must be spaced adequately to prevent overlapping zones. Solvent and growth controls are eessential.

4.3 Broth macrodilution and microdilution

Principle: A series of two-fold (or otherwise defined) concentrations of the test agent are prepared in liquid growth medium and inoculated with a standardised microbial suspension. Growth is then assessed visually, spectrophotometrically or by a validated indicator. Macrodilution is carried out in tubes or similar vessels whereas microdilution is carried out in

microtitre plates and allows for the efficient testing of a range of concentrations and replicates.

Procedure: Prepare a stock extract with a suitable solvent or dispersant; make serial dilutions in broth; include a growth control, a sterility control, a solvent control and a positive antimicrobial control; add the inoculum to give a defined final cell density; incubate under organism-appropriate conditions; and determine the lowest concentration that prevents visible growth. With orange peel the intrinsic colour, turbidity, oil droplets and precipitation can all interfere with the optical or visual endpoint. Extract-only blanks at each concentration and a viable subculture will greatly help to resolve any ambiguity.

The minimum inhibitory concentration (MIC) is the lowest concentration tested which prevents the defined visible growth under the stated conditions. It is not necessarily the concentration which kills the organism. MICs should be reported in standard units (e.g. µg/mL or mg/mL) and the range of concentrations tested should be given. If the inhibition is partial or the endpoint is not clear, the endpoint rule should be described rather than a value selected retrospectively [16,17].

4.4 Minimum bactericidal and fungicidal concentrations

Following MIC testing, aliquots from the wells or tubes which show no visible growth are subcultured onto agent-free agar. The MBC is the lowest concentration which achieves the prespecified bactericidal effect (the MFC being the analogous term for fungi). A commonly used operational definition of the MBC is a 99.9% reduction in viable count (though the exact volume of subculture, the carry-over control and the definition of the endpoint must be specified). Carry-over of drug or extract onto the agar can result in a false suppression of growth and this must be dealt with by dilution, washing or validated neutralisation.

4.5 Agar dilution

Principle: Defined concentrations of the antimicrobial are incorporated into the agar plates. A standardised inoculum is then applied to the surface of the agar (often via a multipoint inoculator) and growth is compared to a drug-free control. Agar dilution is useful for the testing of a number of isolates against a concentration series and is the reference method for a number of bacterial susceptibility applications. Hydrophobic oils may separate, volatilise or distribute in an uneven manner in the agar so the physical stability of the orange peel preparation must be demonstrated. CLSI M07 describes the reference dilution procedures for aerobic bacteria [20].

4.6 Gradient diffusion

A plastic strip with a continuous concentration gradient is placed onto an inoculated agar plate. After incubation the resulting elliptical inhibition zone will intersect the strip at a concentration which can be read as an MIC estimate. Gradient strips are very convenient but do require validated strips, media and reading rules. They are designed for specific antimicrobials and specific organisms. It should not be automatically assumed that they are suitable for use with crude orange peel extracts as the stability of the gradient and the diffusion characteristics may not be appropriate.

4.7 Time-kill assays and bactericidal kinetics

Time-kill testing measures the viable microbial count at a number of time points following exposure to one or more concentrations of the test substance. Samples are removed at defined time intervals, diluted or neutralised to prevent carry-over, plated and counted as colony forming units per ml (CFU/ml). The resulting log10 CFU/ml versus time curve allows distinguishing between rapid killing, delayed killing, regrowth and concentration dependent effects. It is more informative than a single MIC endpoint if the question addressed is that of rate and duration of killing. It is of course a very labour intensive test and is very sensitive to sampling and plating error [16].

4.8 Post-antibiotic effect

The post-antibiotic effect (PAE) is the period of suppression of microbial regrowth following a short period of exposure to an antimicrobial agent and subsequent removal or substantial dilution of that agent. It is a phenomenon that is routinely looked at in the context of antibiotics, though an adapted protocol could be used for defined orange peel fractions. It is essential that the protocol confirms the agent has in fact been removed or neutralised, otherwise any residual compound would be incorrectly interpreted as a persistent biological effect. PAE data are not in any way a substitute for MIC or time-kill data.

4.9 Biofilm assays

Biofilms are surface associated microbial communities embedded in an extracellular matrix. A common screening approach is to use a microtitre plate: the organism is allowed to attach and form a biofilm, the non-adherent cells are washed away and the biomass is stained (e.g. with crystal violet). The stain is then solubilised and the amount measured spectrophotometrically. This provides a measure of total attached biomass rather than viable cells. Viable counts, metabolic dyes, confocal microscopy and matrix analysis are all useful complementary endpoints.

Two quite different questions need to be addressed: i) prevention of biofilm formation in the first place when the test compound is present during the attachment phase; and ii) eradication or reduction of an established biofilm following maturation. The MBC and MBC-E are operationally defined and the methods used to determine them will vary. A reduction in crystal violet staining should not be reported as bactericidal activity without a viability assay.

4.10 Checkerboard and synergy testing

The checkerboard method involves testing two agents across a two-dimensional concentration matrix. The FICI (fractional inhibitory concentration index) is calculated as: FICI = (MIC of A in combination / MIC of A alone) + (MIC of B in combination / MIC of B alone). The cut-offs for interpreting the results as synergy, additivity, indifference and antagonism are not the same in all papers and must be stated. Checkerboard results are screening data and should be confirmed by time-kill studies. For orange peel, combinations could be a chemically defined fraction with an antibiotic, but solvent and dispersant controls are still required.

4.11 TLC bioautography

Thin layer chromatography (TLC) separates the extract constituents on a stationary phase. The plate is developed and dried and then contacted with or sprayed with a microbial suspension or a suitable viability indicator. Clear or non-staining areas may indicate chromatographic zones associated with inhibition. Bioautography is useful in linking activity to the fractions prior to isolation but is of a semi-quantitative nature and is dependent upon compound transfer, residual solvent and the conditions of microbial growth.

4.12 Automated, optical and molecular methods

Automated growth analysers, microplate readers and kinetic optical density systems can monitor growth either continuously or at fairly frequent intervals. Resazurin or tetrazolium indicators can provide a metabolic readout but orange peel pigments and reducing agents can chemically interact with the dyes. Flow cytometry using membrane integrity dyes can allow separation of some of the viable, injured and membrane compromised cells. Fluorescence microscopy, electron microscopy and ATP assays all provide further mechanistic evidence.

Molecular techniques such as qPCR, reverse-transcription qPCR, transcriptomics and proteomics can be used to look at stress responses, virulence genes and pathway changes. The mere detection of nucleic acid does not prove viability as DNA can persist after cell death. Molecular assays should be used in conjunction with culture-based or otherwise validated viability methods. The more sophisticated the approach the greater the mechanistic insight that can be obtained, albeit at the cost of requiring specialist equipment and careful valid

4.13 In vitro and ex vivo models.

There are various in vitro models available, for example, planktonic cultures, biofilm models, co-cultures of epithelial cells, and simulated gastrointestinal fermentation systems. Ex vivo models can range from the use of excised tissue or fresh biological material (appropriate protocols). This can offer a link between simple broth in vitro tests and more complex whole animal testing. It does not allow for host immunity, metabolism, absorption, or tissue distribution. Human-derived samples will require institutional ethics approval, informed consent (if applicable), secure handling, and privacy.

5. FACTORS AFFECTING ANTIMICROBIAL ASSAY RESULTS

Inoculum size. Large inocula can cause apparent MICs to rise (or zone sizes to fall). Conversely, small inocula may give the opposite (exaggerated activity). There should be a defined inoculum and this should, where possible, be checked by viable count. In the case of most bacterial tests the standard is the 0.5 McFarland. The final assay inoculum will depend on the method and the organism [19-21].

Culture media/pH – the effect of composition of the media (such as cation content, thymidine, protein, salts etc.) or pH may have an impact on growth of micro-organisms or activity of antimicrobials. Most non-fastidious aerobic bacteria require (for standard AST) Mueller-Hinton medium. However, there are particulars for particular organisms. It is important to note which medium is used for plant extracts and that it is suitable for the plant extract being tested, as changing the medium may change the MIC reading [19-22].

Incubation conditions (temperature, atmosphere, duration, humidity) may affect growth and endpoint reading. Different organisms have different requirements (bacteria, yeast, moulds). It is important to select suitable conditions for the organism and according to the method standard. It is not a scientifically valid approach to use a single universal condition for incubation.

Solvent/dispersant: The use of ethanol, methanol, dimethyl sulfoxide (DMSO), Tween-type surfactants, or other emulsifiers could cause inhibition of micro-organisms or changes in membrane permeability. A matching vehicle control at the final concentration used in the test must be used. This inhibition must not be due to the dispersant.

Diffusion, Volatility, and Solubility: Hydrophobic terpenes may have very low diffusion in agar, may get into the plastic, may form droplets, and may evaporate. Thus, the apparent zone can both underestimate and overestimate biological activity. Closure, proper mixing, compatible microplates, and broth/agar microdilution confirmation may be needed [8,9].

Composition and Endpoint of the Extract: Crude extracts can be colored, turbid, and viscous, and can also be chemically reducing. Use appropriate concentration matching of extract blanks. Use orthogonal endpoints. Use clear rules for the endpoints. State whether you are getting total visible inhibition or your defined % decrease at your MIC. Do not compare results between papers without considering units, extraction yield, and chemical composition. Quality assurance. Authenticate the plant. Document the extraction. Use pure culture and use reference strains. Use positive, negative, growth, and sterility controls. Make independent biological replicates. Document instrument calibration. Report deviations. Note that for a single culture, replicates in multiple wells are not independent biological replicates (technical replicates). A good study should be able to distinguish between the two.

6. MICROORGANISM SELECTION AND BIOSAFETY

A rational panel of test organisms should cover organisms relevant to the application. It should include a variety of cell envelope types. Typical research organisms could be Gram-positive (e.g. S. aureus, E. faecalis) and Gram-negative (e.g. E.

Coli, P. aeruginosa) bacteria, yeasts (e.g. C. albicans), selected filamentous fungi (e.g. Aspergillus spp.). Selection of the organisms should be driven by intended product, not by convenience. Proper identification and corresponding consent for clinical isolates are required. The support for reproducibility comes from using well-known reference strains from known culture collections. Such reference strains are also used for method quality control. The specific antibiotic susceptibility testing procedures have defined quality control organisms and the acceptable ranges defined by CLSI and EUCAST. However, these ranges are not applicable to orange peel extracts. For natural products, laboratories need to develop their own in-house reproducibility. They will have to report the reference strains and the conditions for the passage. The clinical breakpoints are drug/organism specific. Do not arbitrarily establish breakpoints for plant extracts [19–23].

Nature of organism, route of exposure, local regulations and institutional risk assessment should determine biosafety level and containment. Activities with clinical pathogens should be carried out in appropriately equipped and authorised laboratories, by trained personnel. Appropriate personal protective equipment, biological safety cabinets as indicated, validated decontamination, sharps disposal, and waste disposal are required. Ethics oversight will be needed for human derived ex vivo material. This review does not grant permission to culture pathogens outside an approved laboratory.

7. The gut and intestinal microbiota

7.1 Pectin and fermentable dietary fibre

Pectin is a complex, partially methyl-esterified polysaccharide with a high proportion of galacturonic acid residues. It is a major constituent of plant cell walls and is particularly abundant in citrus albedo. It is not completely digested by the human enzymes in the small intestine and some of it does reach the colon where it can be fermented by the microbial enzymes. The fermentation products (metabolites) include the short-chain fatty acids (SCFAs) acetate, propionate and butyrate and a number of others which can in turn have an effect on the energy supply of the colonic epithelium, the pH of the lumen and host signalling [10,11,12].

Pectooligosaccharides (POS) produced by controlled hydrolysis of pectin are of interest as potential prebiotic substrates. The changes in microbial community profile and fermentation products in an in vitro continuous colon model using faecal inocula from elderly donors is shown for POS derived from lemon peel. Increased levels of the selected beneficial taxa and increased production of SCFA compared to the comparator substrates are reported, providing evidence for the efficacy of the oligosaccharide derived from citrus pectin. This is not a direct human clinical study using sweet orange peel [10].

7.2 Polyphenols and the process of microbial biotransformation

The intact flavanone glycosides may be poorly absorbed and may in fact reach the lower part of the GI tract where the sugar moieties are removed by the action of microbial enzymes. The aglycones that remain are then degraded to smaller phenolic metabolites. The resulting metabolites may have very different bioavailability and biological activity to the parent compounds. Polyphenols can also have a specific effect on the growth or metabolic activity of microbes in vitro. The net effect of these factors will be a function of the dose, the food matrix, the host physiology and the baseline microbiota [11-13].

7.3 Gut barrier and inflammatory signalling

It is well established that the fermentation products of pectin (e.g. butyrate) are important for energy metabolism and for the maintenance of the epithelial barrier. The effect of citrus polyphenols on inflammatory signalling and oxidative stress has been investigated. It would be incorrect to conclude that eating orange peel will treat IBD, dysbiosis or infection. Any plausible mechanism of action and/or animal or in vitro data would need to be validated in a human study in a controlled setting with the specific ingredient and the clinically relevant endpoint.

7.4 Terminology and evidence of prebiotics

A prebiotic is a substrate that is selectively utilised by the host micro-organisms and which confers a health benefit. Simply being a fermentable fibre is not in itself a prebiotic – there has to be evidence of selectivity for utilisation and evidence of a beneficial effect. For orange-peel-derived ingredients there would need to be evidence of compositional characterisation, resistance to digestion, fermentation, effect on the microbiota and a host-relevant beneficial effect. A synbiotic formulation would require a live micro-organism and a substrate which is selectively utilised by the host micro-organism – there would need to be evidence of compatibility and viability.

Table 2. Orange-Peel Components and Possible Gut-Health Roles.

Component	Potential intestinal role	Evidence status / caution
Pectin	Fermentable fibre; substrate for microbial fermentation and short-chain fatty acid (SCFA)generation	Strong mechanistic rationale; orange-specific human outcomes remain limited
Pectooligosaccharides	More accessible fermentable oligosaccharides; may influence gut microbial community composition	In vitro citrus-peel models support potential; not proof of clinical benefit
Hesperidin / narirutin	Microbial deglycosylation and conversion to phenolic metabolites	Metabolism is plausible and studied; response varies by microbiota
Polymethoxyflavones	Potential modulation of microbial and inflammatory pathways	Mostly preclinical; dose and bioavailability need clarification
Essential oils	Antimicrobial effects may influence selected intestinal microbes	Concentrated oil can irritate tissues; oral safety and microbiome selectivity need assessment

8 PHARMACEUTICAL APPLICATIONS

8.1 Standardised phytopharmaceutical extracts

A standardised orange peel extract could be developed on the basis of defined marker compounds (e.g. hesperidin, narirutin or total phenolics). Standardisation would need to cover botanical identity, extraction yield, chromatographic fingerprint, limits for residual solvents, pesticides, heavy metals, microbial load and stability. Marker content alone may not

be sufficient to ensure biological consistency if the active principle is a mixture in which case a chemical fingerprint and a relevant bioassay would be required.

8.2 Topical antimicrobial and wound-care formulations

Orange peel essential oil or selected phenolic fractions could be evaluated in the form of gels, creams, sprays, films or wound dressings. The formulation would need to take into account volatility, oxidation, odour, water solubility, irritation and controlled release. Antimicrobial activity would have to be confirmed in the final formulation rather than just in the initial extract. Cytotoxicity (against keratinocytes or fibroblasts), irritation, sensitisation, stability and release testing would all be important prior to considering any wound care claims.

8.3 Pectin as a pharmaceutical excipient

Citrus pectin has a well-established technological role as a gelling, thickening and stabilising agent. It has been the subject of pharmaceutical research in several forms including beads, hydrogels, films, microparticles and colon-targeted delivery systems. It can be used to form gels via ionic cross-linking or other formulation approaches. The rate of drug release is a function of the pectin's swelling properties, viscosity, degree of esterification, molecular weight and its interaction with co-polymers. Orange peel pectin may be a source of value, but of course it would have to meet the requisite specifications for purity, microbiology and performance.

8.4 Encapsulation and nanoformulations

Encapsulation can be used to improve the handling of hydrophobic essential oils and to protect oxidation-sensitive compounds. Emulsions, nanoemulsions, liposomes, polymeric nanoparticles and biopolymer matrices have all been investigated for citrus-derived materials. The potential benefits are improved dispersion, reduced volatilisation, controlled release and better contact with the microbial surface. The benefits will be formulation-specific and will need to be demonstrated via particle size analysis, polydispersity, zeta potential where appropriate, encapsulation efficiency, release kinetics, stability and biological testing.

8.5 Green synthesis and biomaterials

Citrus peel extracts contain reducing and capping agents which have been used in the green synthesis of metal and metal oxide nanoparticles. Citrus waste-derived bionanomaterials have been suggested for use in antimicrobial coatings, wound care and drug delivery [18]. The nanoparticles themselves (not just the extract) need to be characterised for size, morphology, composition, residual reagents, dissolution and toxicity. Green synthesis does not equate to being safe or biocompatible.

8.6 Food, nutraceutical and cosmeceutical applications

Orange peel ingredients can be used in functional foods, fibre-fortified products, natural preservatives, oral care products and cosmetic preparations. The regulatory classification will be dependent on the intended use, the composition, the claims and the jurisdiction. A food or cosmetic ingredient cannot be marketed as a medicine without meeting the appropriate regulatory requirements. The line between a nutraceutical claim and a therapeutic indication must be drawn.

The pharmaceutical applications of orange-peel components are summarised in Table 3.

Table 3. Potential Pharmaceutical Applications of Orange Peel.

Pharmaceutical application	Orange peel component	Potential application and benefits
Drug delivery systems	Pectin	Potential use as a natural polymer in controlled and sustained drug-release formulations
Microencapsulation	Pectin and other polysaccharides	Encapsulation and protection of sensitive bioactive compounds
Pharmaceutical excipients	Pectin and cellulose	Potential use as natural formulation materials, subject to functionality and quality evaluation
Nanotechnology	Citrus-derived phytochemicals	Potential use in green synthesis of nanoparticles for biomedical research
Antimicrobial formulations	Essential oils and flavonoids	Investigation as bioactive ingredients in antimicrobial preparations
Nutraceutical formulations	Flavonoids, carotenoids and dietary fibre	Development of functional food and nutraceutical products

9. ADVANTAGES AND LIMITATIONS

9.1 Advantages

Orange peel is readily available, renewable and potentially low-cost. It contains several classes of bioactive compounds and useful polysaccharides enabling a range of product streams from a single feedstock. Its valorisation would lead to a reduction in waste and

support the circular economy. Pectin has good formulation properties and the essential oil and phenolic fractions provide a basis for antimicrobial and antioxidant research [3,4,16].

9.2 Limitations

The principal drawbacks are batch-to-batch compositional variability, incomplete identification of the active constituents, poor solubility of the essential oils, antimicrobial results which are assay-dependent, potential for cytotoxicity and sensitisation, uncertain oral bioavailability and a lack of robust human clinical data. Natural does not equal safe. Concentrated essential oils can be irritating and interactions with drugs or with altered metabolism should be considered rather than assumed to be non-existent.

A further caveat is evidence transfer. Results from lemon, mandarin or mixed citrus peels cannot be taken as direct evidence for C. sinensis. In vitro antimicrobial inhibition does not prove that an ingredient given orally would reach an infected tissue at an effective concentration. PK, toxicology, formulation and clinical validation are all required.

10. RECENT ADVANCES AND DEVELOPMENTS

Recent research is focused on greener extraction, integrated recovery of oil and pectin, chromatographic metabolomics, microbiome-based fibre ingredients and encapsulation of volatiles. Ultrasound and microwave-assisted extraction can reduce processing times in some cases, with the caveat that optimisation is required to avoid degradation of the thermolabile components. Enzyme-assisted extraction can be used to modify the cell wall structure and enhance the recovery of certain fractions [3,4,16].

Citrus flavonoids remain of interest as antimicrobial leads with several recent reviews summarising the evidence for hesperidin, the naringenin-related compounds and the polymethoxyflavones [15]. Orange peel-focused gut health reviews have also looked at the preclinical and clinical ingredient research but note that the evidence is highly heterogeneous and that not all of the commercial preparations are equivalent to the whole orange peel [12,13].

For antimicrobial method development the current reviews stress the importance of reporting the chemical composition of the essential oils, the use of a range of complementary assays, stating solvent and emulsifier concentrations, expressing MICs in standard units and carrying out independent replicates [8,9,16]. More sophisticated techniques such as flow cytometry, imaging and molecular profiling can be used to help elucidate the mechanism of action but should be used to complement rather than replace the viable count and quantitative growth endpoints.

11. CONCLUSION

Future work should start with properly authenticated, traceable C. sinensis material and evaluate the flavedo and albedo separately rather than assuming the peel is chemically homogeneous. The extraction should be optimised for the particular fraction being studied and each batch should be fingerprinted by HPLC/LC–MS or GC–MS. Marker compounds, extraction yield and residual contaminants should all be reported.

Antimicrobial studies should be staged: initial diffusion or bioautography; quantitative broth microdilution with appropriate vehicle controls; MBC/MFC confirmation; time-kill testing and biofilm or synergy studies only where there is a genuine rationale for doing so. Results should be reported in mass-based units with strain IDs, replicate structure, raw endpoint definitions and chemical composition. Cytotoxicity and selectivity indices should be determined prior to any proposal for topical or systemic therapy.

Gut health work needs to isolate the pectin and oligosaccharide fractions, define the molecular weight and esterification, look at the simulated digestion and fermentation and then proceed to well-controlled human studies. The microbiome changes need to be linked to the metabolites and validated by actual health outcomes. The effect of essential oils on the commensal organisms and mucosal safety is requires investigation.

Pharma translation will depend on reproducible manufacturing, stability, quality specs, safety, regulatory status and real-world cost. Formulation development needs to consider the whole product rather than simply assume that the activity of the raw extract will carry through into the final product. Wherever one is using a nanoparticle or encapsulation system you need to examine long-term stability, release, biodistribution and toxicology.

A key research priority is to separate biological activity from therapeutic utility. Lab inhibition is a discovery signal, it is not in itself evidence of a clinically useful antimicrobial. Clinical efficacy will only be demonstrated by an achievable exposure at the site of infection, an acceptable safety margin, reproducible manufacturing and appropriate human studies.

12. Future Perspectives

The authors may mention their institution, their advisor, their lab group or their funder where appropriate. Any institution, funding body or individual should not be credited for support that they in fact did not receive.

13. REFERENCES

1. Liu N, Li X, Zhao P, Zhang X, Qiao O, Huang L, Guo L, Gao W. A review of chemical constituents and health-promoting effects of citrus peels. Food Chem. 2021 Dec 15;365:130585. Doi: 10.1016/j.foodchem.2021.130585. Epub 2021 Jul 13. PMID: 34325351.
2. M'hiri, N., Ioannou, I., Ghoul, M., & Mihoubi Boudhrioua, N. (2017). Phytochemical characteristics of citrus peel and effect of conventional and nonconventional processing on phenolic compounds: A review. Food Reviews International, 33(6), 587–619. https://doi.org/10.1080/87559129.2016.1196489
3. Putnik P, Bursać Kovačević D, Režek Jambrak A, Barba FJ, Cravotto G, Binello A, Lorenzo JM, Shpigelman A. Innovative "Green" and Novel Strategies for the Extraction of Bioactive Added Value Compounds from Citrus Wastes-A Review. Molecules. 2017 Apr 27;22(5):680. Doi: 10.3390/molecules22050680. PMID: 28448474; PMCID: PMC6154587
4. Sharma K, Mahato N, Cho MH, Lee YR. Converting citrus wastes into value-added products: Economic and environmently friendly approaches. Nutrition. 2017 Feb;34:29-46. Doi: 10.1016/j.nut.2016.09.006. Epub 2016 Sep 30. PMID: 28063510.
5. Durmus N, Gulsunoglu-Konuskan Z, Kilic-Akyilmaz M. Recovery, Bioactivity, and Utilization of Bioactive Phenolic Compounds in Citrus Peel. Food Sci Nutr. 2024 Nov 7;12(12):9974-9997. Doi: 10.1002/fsn3.4570. PMID: 39723030; PMCID: PMC11666827.
6. Oikeh, E.I., Oviasogie, F.E. & Omoregie, E.S. Quantitative phytochemical analysis and antimicrobial activities of fresh and dry ethanol extracts of Citrus sinensis (L.) Osbeck (sweet Orange) peels. Clin Phytosci 6, 46 (2020). https://doi.org/10.1186/s40816-020-00193-w
7. Ciriminna, R., Petri, G.L., Angellotti, G., Luque, R., Fabiano Tixier, A.-S., Meneguzzo, F. and Pagliaro, M. (2025), Citrus Flavonoids as Antimicrobials. Chem. Biodiversity, 22: e202403210. https://doi.org/10.1002/cbdv.202403210
8. Hulankova, R. Methods for Determination of Antimicrobial Activity of Essential Oils In Vitro—A Review. Plants 2024, 13, 2784. https://doi.org/10.3390/plants13192784
9. Bubonja-Šonje M, Knežević S, Abram M. Challenges to antimicrobial susceptibility testing of plant-derived polyphenolic compounds. Arh Hig Rada Toksikol. 2020 Dec 31;71(4):300-311 Doi: 10.2478/aiht-2020-71-3396. PMID: 33410777; PMCID: PMC7968511.

10. Míguez B , Vila C , Venema K , Parajó JC , Alonso JL . Prebiotic effects of pectooligosaccharides obtained from lemon peel on the microbiota from elderly donors using an in vitro continuous colon model (TIM-2). Food Funct. 2020 Nov 18;11(11):9984-9999. Doi: 10.1039/d0fo01848a. PMID: 33119011.
11. Gu Q, Gao X, Zhou Q, Li Y, Li G, Li P. Characterization of soluble dietary fiber from citrus peels (Citrus unshiu), and its antioxidant capacity and beneficial regulating effect on gut microbiota. Int J Biol Macromol. 2023 Aug 15;246:125715. Doi: 10.1016/j.ijbiomac.2023.125715. Epub 2023 Jul 5. PMID: 37419261.
12. Ademosun AO, Ajeigbe OF, Ademosun MT, Ogunruku OO, Oboh G. Improving gut microbiome through diet rich in dietary fibre and polyphenols: the case for orange peels. Human Nutrition & Metabolism. 2025;39:200295. https://doi.org/10.1016/j.hnm.2024.200295
13. Vilas-Boas, A.A.; Correia, M.; Campos, D.A.; Pintado, M. Upcycled Orange Peel Ingredients for Gastrointestinal and Cardiometabolic Health: A Scoping Review and Market Perspectives. Nutrients 2026, 18, 1126. https://doi.org/10.3390/nu18071126
14. Gonzalez-Pastor, R.; Carrera-Pacheco, S.E.; Zúñiga-Miranda, J.; Rodríguez-Pólit, C.; Mayorga-Ramos, A.; Guamán, L.P.; Barba-Ostria, C. Current Landscape of Methods to Evaluate Antimicrobial Activity of Natural Extracts. Molecules 2023, 28, 1068. https://doi.org/10.3390/molecules28031068
15. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016 Apr;6(2):71-79. Epub 2015 Dec 2. PMID: 29403965; PMCID: PMC5762448.https://doi.org/10.1016/j.jpha.2015.11.005
16. Hossain TJ. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. Eur J Microbiol Immunol (Bp). 2024 Apr 22;14(2):97-115. Doi: 10.1556/1886.2024.00035. PMID: 38648108; PMCID: PMC11097785.
17. AlZahabi S, Mamdouh W. Valorization of citrus processing waste into high-performance bionanomaterials: green synthesis, biomedicine, and environmental remediation. RSC Adv. 2025 Oct 3;15(43):36534-36595. Doi: 10.1039/d5ra04307g. PMID: 41048549; PMCID: PMC12495416.
18. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. 14th ed. CLSI standard M02. Wayne (PA): Clinical and Laboratory Standards Institute; 2024.

19. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 12th ed. CLSI standard M07. Wayne (PA): Clinical and Laboratory Standards Institute; 2024.
20. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 36th ed. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute; 2026.
21. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Disk diffusion and quality control. EUCAST disk diffusion method manual. Version 13.0. January 2025.
22. International Organization for Standardization (ISO). Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. ISO 20776-1:2019. Geneva: ISO; 2019.